activity in a subject. In different claims, the invention encompasses *in vivo* and *in vitro* methods of monitoring the activity of PKC-epsilon and determination of the anxiety modulation in a subject. The Examiner maintains that the specification is not enabling for the claimed method because the specification has not disclosed sufficient guidance to make and practice the claimed method in a subject and an artisan of skill would have required undue experimentation to practice the claimed method. Paper 10, page 3, lines 9-18.

The Examiner divides his arguments for rejection under 35 U.S.C. 112, ¶ 1, into several sections, each of which will be addressed in sequence below. The Examiner first asks: what is the method of testing the PKC-epsilon modulatory activities of a compound; would it be determined in *in vitro* or in *in vivo* system? (Paper 10, page 3, lines 19-20). The answer is that the method of testing the PKC-epsilon modulatory activities of a compound may be determined both *in vitro* and *in vivo*, as claimed in the instant invention. Claim 32, and its dependent claims 33 and 34, are drawn to an *in vitro* method of testing. Claim 35, and its dependent claims 36-39, are drawn to a combination of *in vitro* and *in vivo* screening, and Claim 10, and its dependent claims 29-31, are drawn to an *in vivo* method.

The Examiner asks the further question of whether the results obtained in *in vitro* systems can be extrapolated to *in vivo* systems or can an effect on the activity of PKC-epsilon be reproduced *in vivo* when the same compound is given to an animal *in vivo.* (Paper 10, page 3, lines 26-28) Applicant respectfully suggests that this question is not applicable to the claims as written. It is known to those skilled in the art that is impossible to test the *activity* of PKC-epsilon directly in a live animal, as activity measurements are conventionally done *in vitro* with enzymatic activation, ligand or substrate binding, or cellular assays. Claim 32 is clearly drawn to such *in vitro* test methods. Claims 10 and 35, when referring to *in vivo* testing, are drawn to measuring *symptoms of anxiety* in test animals and not to measuring the activity of PKC-epsilon.

Applicant respectfully disagrees with the Examiner's contention (Paper 10, page 4, lines 5-6) that it is not clear how the effects of a compound on the activity of PKC-

epsilon can be considered an indication of its anxiety modulatory activity. As the Examiner notes, methods of identifying PKC-epsilon inhibitors *in vitro* are well-known to those skilled in the art. However, before the instant invention, it was not known that such compounds could be used useful in the treatment of anxiety. Applicant has well proven the link between modulation of PKCε activity and an effect on anxiety as set forth in the specification by subjecting $PKC\varepsilon^{-/-}$ mice to standard tests for anxiety, showing them to have less fear and anxiety than wild-type mice. (For supporting data, see Examples 3, 6, 7, 8, 11 and 12 in the specification.) These data clearly show that test subjects without PKC-epsilon do not become anxious to the extent that normal, wild-type subjects do when subject to the same stressors.

Additional data in support of a link between PKCε activity and anxiety are given in the attached Declaration under 37 C.F.R. 1.132 by co-inventor Robert O. Messing, M.D. The data presented in Figure 1 of the Declaration and described in paragraph 3 show that $PKC\varepsilon^{-/-}$ mice ("PKCε null mice") created from different genetic strains of mice exhibit less anxiety as opposed to normal, wild-type mice. The Examiner has expressed discomfort with the differences observed between male mice and female mice in the data presented in the application. Paragraph 2 of the Declaration addresses these differences and specifies that only male subjects were used in further experiments, as is commonly done by those skilled in the art.

The Examiner asks two further questions: 1) can the activity of a compound be tested for PKC-epsilon modulation in the mutant mouse disclosed in the specification? (Paper 10, page 4, lines 27-28); and what is a subject, who can be used for determining efficacy of a compound in modulating the state of anxiety? (Paper 10, page 5, lines 9-10). These questions may conveniently be handled together. Applicant assumes that the Examiner is referring to claims 10, 29-31 and lines 5-9 of claim 35, which are drawn to a method of identifying compounds that modulate anxiety involving a test subject or animal. As explained below, Applicant has amended claims 10, 29-31, and 35-39 in response to the Examiner's rejection under 35 U.S.C. 112, ¶ 2. These claims now clearly state that test compounds are administered to a test animal that is subject to anxiety. Although it would be well-known to one skilled in the art that a test subject susceptible to

the condition being tested should be employed in a test method, Applicant agrees that this point may be clarified and has done so by amendment of the claims. Therefore, the method of claims 10, 29-31 and lines 5-9 of claim 35 is carried out *in vivo* and the subject is a test animal that is subject to anxiety. As to whether the PKC$\varepsilon^{-/-}$ mice described in the specification may be used as test subjects, it should thus now be clear that these mice are not useful test subjects, whereas, for example, wild-type animals are. The inclusion of the description of the PKC$\varepsilon^{-/-}$ mice in the specification was not to suggest that they are useful as test subjects for the method of the invention, but instead to prove that there is a link between modification of PKC-epsilon activity and modulation of anxiety in a whole animal.

Regarding the Examiner's specific rejection of claim 31, Applicant is confused by the Examiner's statement about specific characteristics of a test subject:

> In claim 31, the method indicates that the symptoms of anxiety can be decreased locomotor activity, decreased time in open areas, decreased exploratory behavior, and increased basal level of a stress hormone. However, the specification does not disclose that a subject should have these characteristics to qualify as a subject. Paper 10, page 5, lines 16-19.

In fact, these behaviors are not innate characteristics of desirable test subjects. The screening method of the claimed invention is a *comparative* test and not a measurement of absolute intrinsic characteristics. Further, Applicant does disclose the enumerated behaviors as measurable symptoms of anxiety in anxiety-prone test subjects in Example 3 and 11. A test subject *should not* exhibit these behaviors in the normal (non-stressed) state, but should only exhibit them when stressors are applied as in Examples 3 and 11. This concept is well-known to one skilled in the art for both the elevated plus maze test [S. Hogg, *Pharmacol. Biochem. Behav.* (1966) 54:21-30; copy attached] and the measurement of stress hormones before and after application of a stressor [R. L. Hauger *et al.*, *Endrocrinology* (1988) 123:396-405; copy previously provided with IDS]. Applicant therefore respectfully disagrees that claim 31 is not enabled by the specification.

The Examiner cites a paper by Rogers *et al.*, which describes behavioral differences in different strains of mice, and maintains that this paper is evidence "that any mice may not be used for comparing phenotypes of mice" because changes in animal behavior may be due to natural variation (Paper 10, page 6, lines 7-13). Applicant respectfully submits that this is not a valid argument for the reasons set forth above regarding the measurement of relative changes in behavior. Further, as described in paragraph 3 of the attached inventor's Declaration, additional experiments show that although there are other genetic differences between hybrid mice and inbred mice, the effect of the PKC$\varepsilon^{-/-}$ mutation can be discerned.

In response to the Examiner's rejection of claim 29, Applicant has amended claim 29 to include the limitation of claim 30. Claim 30 has therefore been cancelled. Regarding the Examiner's rejection of claim 34, Applicant has rendered this rejection moot by cancellation of claim 34.

For the reasons described in the preceding sections, the Applicant respectfully requests withdrawal of the rejection of claims 10, and 29-39 under 35 U.S.C. §112, first paragraph.

II. DEFINITENESS

The Examiner has rejected claims 10, 29, 30, 31 and 35 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner states that claims 10, 29 and 30 lack antecedent basis for the limitation "the symptoms of anxiety" and that claim 35 is vague because of the use of the same phrase. Applicant thanks the Examiner for his point and has amended claims 10, 29 and 30 to remove the indefinitiveness. No new matter is added by these amendments. Support for these claim amendments are found throughout the specification and, for example, on page 26, lines 9-25, and page 27, lines 11-17.

The Examiner maintains that claim 31 is vague and indefinite because it is unclear as to what is meant by the term "stress hormone." Applicant respectfully points out that examples of stress hormones (corticosterone and adrenocorticotropic hormone) are given

in the specification on page 26, lines 20-23. This passage also gives a description of the effect of these hormones on test subjects. Applicant therefore requests that the rejection of claim 31 for indefiniteness be withdrawn.

Accordingly, in view of the above remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (650) 843-5214.

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Attachments

VERSION WITH MARKINGS TO SHOW CHANGES

10. A method of identifying a compound that modulates anxiety, said method comprising:

selecting, as a test compound, a compound that modulates the activity of PKCε, and

administering said test compound to a test animal subject to anxiety to determine whether said [the symptoms of] anxiety is [are] modulated.

29. The method of claim 10, wherein said compound selectively inhibits the activity of PKC epsilon and said [symptoms of] anxiety is [are] reduced.

Cancel claim 30.

31. The method of claim 29 [10], wherein said reduction of anxiety in a test animal is [symptoms of anxiety are] selected from the group consisting of: decreased locomotor activity, decreased time in open areas, decreased exploratory behavior, and increased basal level of a stress hormone.

32. (Reiterated) A method of identifying compounds that modulate anxiety, said method comprising:

exposing a functional PKC epsilon to a test compound,

determining whether the test compound modulates the activity of PKC epsilon, wherein test compounds that modulate the activity of PKC epsilon are identified as compounds for modulating anxiety.

33. (Reiterated) The method of claim 32, wherein said exposing is performed in a cell or cell lysate.

Cancel claim 34.

35. A method of identifying compounds that modulate anxiety, said method comprising:

measuring the activity of PKC epsilon in the presence and absence of a test compound,

determining whether the test compound modulates the activity of PKC epsilon,

administering to an animal subject to anxiety a test compound that modulates the activity of PKC epsilon and

determining whether the animal's [symptoms of] anxiety is [are] modulated, wherein test compounds that modulate the animal's [symptoms of] anxiety are identified as compounds for modulating anxiety.

36. (Reiterated) The method of claim 35, wherein said measuring is performed in a cell or cell lysate.

37. (Reiterated) The method of claim 35, wherein said measuring is performed in an in vitro assay.

38. (Reiterated) The method of claim 35, wherein said administering occurs under conditions in which the animal, in the absence of the test compound, displays symptoms of anxiety.

39. (Reiterated) The method of claim 35, wherein said second determining step is performed after the animal is exposed to an anxiety-producing stimulus.